bio-protocol Preprint

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Whole-mount immunostaining

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An abbreviated version of this protocol was published in eLIFE in Oct 2019

A bidirectional network for appetite control in larval zebrafish

DOI: 10.7554/eLife.43775

Detailed protocol

Whole-mount immunostaining for dissected brains

1. Fix whole fish overnight in 4% PFA (4%PFA, PH7.2-7.4, with 1.2% glucose and 0.34mM CaCl2) at 4 degrees
2. Quick wash with 1XPBT(0.3% triton in 1XPBS) 3 times
3. Dissect brains in 1XPBS
4. Quick wash with 1XPBT once before adding 100-150ul blocking buffer (2%BSA in PBS+0.3% triton), block for 1 hour at 4 degrees
5. Add primary antibodies diluted accordingly in blocking buffer and shake gently on shaker at 4 degrees (duration depends on antibodies, 2 days work better for PERK).
6. Wash with PBT 3 times (10 minutes each)
7. Block with blocking buffer for 1 hour at 4 degrees
8. Add secondary antibodies (diluted in blocking buffer) for 1-2 days at 4 degrees on shaker
9. Wash 3X with PBT
10. Add 70% glycerol in 1XPBS and mount on slides, ventral side up
11. Image with confocal microscope

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Wee, C. , Kunes, S. and Song, E. (2021). Whole-mount immunostaining. Bio-protocol Preprint. bio-protocol.org/prep1115.
2. Wee, C. L., Song, E. Y., Johnson, R. E., Ailani, D., Randlett, O., Kim, J., Nikitchenko, M., Bahl, A., Yang, C., Ahrens, M. B., Kawakami, K., Engert, F. and Kunes, S.(2019). A bidirectional network for appetite control in larval zebrafish. eLIFE. DOI: 10.7554/eLife.43775